PATENT
Customer No. 22,852
Attorney Docket No. 3495-0178-01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	
Ladant et al.	Group Art Unit for parent application: 1645
Serial No.: Not yet assigned	
Filed: Concurrently herewith	Examiner in parent application: Zeman
For: BACTERIAL MULTI-HYBRID SYSTEM AND APPLICATIONS THEREOF	

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

PRELIMINARY AMENDMENT

Prior to the examination of the above application, please amend this application as follows:

IN THE SPECIFICATION:

Please delete the first paragraph of page 1, and insert the following paragraph therefor:

This regular U.S. application is a division of U.S. Application Serial No. 09/203,681 filed December 1, 1998, and claims the benefit of U.S. Provisional patent application serial No. 60/067,308, filed December 4, 1997, the entire disclosure of each of which is relied upon and incorporated by reference herein.

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Page 43, please delete the paragraph beginning at line 11, and insert the following paragraph therefor:

Plasmid pUT18 (3023-bp) is a derivative of the high copy number vector pUC19 (expressing an ampicillin resistance selectable marker and compatible with pT25 or pKT25) that encodes the T18 fragment (amino acids 225 to 399 of CyaA). In a first step, we constructed plasmid pUC19L by inserting a 23-bp double-stranded oligonucleotide (5'-AATTCATCGATATAACTAAGTAA-3' (SEQ ID No.: 1)) and its complementary sequence) between the *Eco*RI and *Nde*I sites of pUC19. Then, a 534-bp fragment harboring the T18 open reading frame was amplified by PCR (using appropriate primers and pT18 as target DNA) and cloned into pUC19L digested by *Eco*RI and *Cla*I (the appropriate restriction sites were included into the PCR primers). In the resulting plasmid, pUT18, the T18 open reading frame is fused in frame downstream of the multicloning site of pUC19. This plasmid is designed to create chimeric proteins in which a heterologous polypeptide is fused to the N-terminal end of T18 (see map).

Please insert the attached Sequence Listing (pages 1-4) as pages 62-65 and renumber the application accordingly.

IN THE CLAIMS:

Please cancel claims 1-9, 24, and 39-45 without prejudice or disclaimer. Please amend claims 10-22 and 25-38 and add new claims 45-48, as follows:

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10. (AMENDED) A method of selecting a molecule of interest, which is capable of binding to a target ligand, wherein the interaction between the molecule of interest and the target ligand is detected with a signal amplification system comprising:

(a) a first chimeric polypeptide comprising a first fragment of an enzyme chosen from adenylate cyclase and guanylate cyclase;

(b) a second chimeric polypeptide comprising a second fragment of an enzyme chosen from adenylate cyclase and guanylate cyclase, or a modulating substance capable of activating adenylate cyclase or guanylate cyclase, and

(c) a signal molecule precursor,

wherein the first fragment is fused to a molecule of interest, and the second fragment or the modulating substance is fused to a target ligand, and wherein the activity of the enzyme is restored by the *in vivo* interaction between the molecule of interest and the target ligand and wherein a signal amplification is generated by the restored enzyme activity; and

wherein the *in vivo* interaction occurs in a bacterial cell.

11. (AMENDED) The method of selecting a molecule of interest according to claim 10, wherein the signal amplification corresponds to production of a signal molecule.

12. (AMENDED) The method of selecting a molecule of interest according to claim 10, wherein the signal amplification triggers transcriptional activation.

13. (AMENDED) The method of selecting a molecule of interest according to claim 10, wherein the signal amplification system comprises a bacterial multi-hybrid system of at least two distinct fragments of an enzyme.

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14. (AMENDED) The method of selecting a molecule of interest according to claim 10, wherein the signal amplification system comprises a bacterial multi-hybrid system of at least a first fragment of an enzyme and a modulating substance.

15. (AMENDED) The method of selecting a molecule of interest according to claim 10, wherein the target ligand is selected from proteins, peptides, polypeptides, receptors, ligands, antigens, antibodies, DNA binding proteins, glycoproteins, lipoproteins, and recombinant proteins.

16. (AMENDED) The method of selecting a molecule of interest according to claim 15, wherein the molecule of interest is capable of interacting with the target ligand.

17. (AMENDED) The method of selecting a molecule of interest according to claim 11, wherein the interaction between the molecule of interest and the target ligand is quantified by measuring synthesis of the signal molecule.

18. (AMENDED) The method of selecting a molecule of interest according to claim 11, wherein the signal molecule comprises cAMP.

19. (AMENDED) The method of selecting a molecule of interest according to claim 11, wherein the signal molecule comprises cGMP.

20. (AMENDED) The method of selecting a molecule of interest according to claim 46, wherein the reporter gene is selected from genes coding for a nutritional marker, genes conferring resistance to antibiotics, genes encoding a toxin, genes encoding a color marker, genes encoding a phase receptor protein or fragment thereof, and any other gene giving a selectable phenotype.

21. (AMENDED) The method of selecting a molecule of interest according to claim 10, wherein the molecule of interest is a mutant molecule compared to a known

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wild type molecule and said molecule of interest is tested for its capacity to interact with the target ligand.

22. (AMENDED) The method of selecting a molecule of interest according to claim 10, wherein the selection is performed in an *E. coli* strain or in any bacterial strain deficient in endogenous adenylate cyclase or guanylate cyclase.

25. (AMENDED) A method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest, wherein the stimulating or the inhibiting is detected with a signal amplification system comprising:

(a) a first chimeric polypeptide comprising a first fragment of an enzyme chosen from adenylate cyclase and guanylate cyclase;

(b) a second chimeric polypeptide comprising a second fragment of an enzyme chosen from adenylate cyclase and guanylate cyclase, or a modulating substance capable of activating adenylate cyclase or guanylate cyclase, and

(c) a signal molecule precursor,

wherein the first fragment is fused to a molecule of interest, and the second fragment or the modulating substance is fused to a target ligand, and wherein the activity of the enzyme is restored by the *in vivo* interaction between the molecule of interest and the target ligand and wherein a signal amplification is generated by the restored enzyme activity; and

wherein the *in vivo* interaction occurs in a bacterial cell, and

wherein the activity of the enzyme is tested in the presence and absence of the test substance.

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26. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 25, wherein the signal amplification system comprises a bacterial multi-hybrid system of at least two distinct fragments of an enzyme.

27. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 25, wherein the signal amplification system comprises a bacterial multi-hybrid system of at least a first fragment of an enzyme and a modulating substance.

28. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 25, wherein the signal amplification corresponds to production of a signal molecule.

29. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 28, wherein the signal amplification corresponding to the production of a signaling molecule is blocked or partially abolished.

30. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 25, wherein the signal amplification generated by the restored enzyme activity leads to transcriptional activation, which leads to reporter gene expression.

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31. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 30, wherein the transcriptional activation leading to reporter gene expression is blocked or partially abolished.

32. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 25, wherein the target ligand is selected from receptors, ligands, antigens, antibodies, DNA binding proteins, glycoproteins and lipoproteins.

33. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 25, wherein the test substance is selected from proteins, glycoproteins, lipoproteins, ligands, and any other compound having stimulating or inhibitory affinity.

34. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 28, wherein the signal molecule corresponds to cAMP.

35. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 28, wherein the signal molecule corresponds to cGMP.

36. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 30, wherein the reporter gene is selected from genes coding for a nutritional marker, genes conferring resistance to antibiotics, genes encoding toxin,

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genes encoding a color marker, genes encoding phage receptor proteins or a fragment thereof, and any other gene giving a selectable phenotype.

37. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 25, wherein the molecule of interest is a mutant molecule compared to a known wild type molecule and said molecule of interest is tested for its capacity of interacting with the target ligand.

38. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 25, wherein the screening is performed in an *E. coli* strain or in any bacterial strain deficient in endogenous adenylate cyclase or guanylate cyclase.

46. (NEW) The method of selecting a molecule of interest according to claim 12, wherein the transcriptional activation leads to expression of a reporter gene.

47. (NEW) The method of selecting a molecule of interest according to claim 46, wherein the interaction between the molecule of interest and the target ligand is quantified by measuring the expression of the reporter gene.

48. (NEW) The method of selecting a molecule of interest according to claim 15, wherein the molecule of interest is capable of interacting with the target ligand and of binding to the target ligand.

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IN THE DRAWINGS:

Please amend Figure 2 and Figure 3.2, and replace Figure 3.1 with the Substitute Figure 3.1, submitted herewith, as indicated in the attached Request for Approval of Drawing Changes.

REMARKS

Claims

This application is a division of U.S. Application Serial No. 09/203,681 filed December 1, 1998. In that application, the Examiner restricted prosecution to Group I, claims 1-9, 23, 24, 39, and 43. With this Preliminary Amendment, the claims of Group II, i.e., claims 10-22 and 25-38, are presented for prosecution. Thus, with this Preliminary Amendment, claims 1-9, 24, and 39-45 are canceled without prejudice or disclaimer. Claims 10 and 25 are rewritten in independent form, new claims 45-48 are added, and the remaining claims are rewritten to correct dependencies and to even more clearly define the invention.

Specification

Applicants have amended the application to include a Sequence Listing in accordance with 37 C.F.R. §§1.821 - 1.825. Applicants have also corrected omissions of Sequence Identifiers in Figures 2, 3.1, and 3.2. No new matter is added by these amendments.

During prosecution of the parent application, inconsistencies were noticed in two areas of the application as filed: page 43 of the specification and Figure 3.1.

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Amendments have been made to clarify these portions of the application, and the following remarks are presented in support of the amendments.

First, it was noticed that in the lower portion of Figure 3.1, the third codon from the end, "TGC" was shown as corresponding to "SER". This correspondence is not proper, as SER is properly coded for by TCG. A typographical error had apparently been made. Substitute Figure 3.1 corrects this inconsistency.

Second, in reviewing the description of plasmid pUT18 at page 43 of the specification, another inconsistency was noticed. The nucleotide sequence shown on lines 16-17 (SEQ ID No. 1) was incorrect because an "A" had been omitted from the 5' end of the sequence. The entire sequence was properly shown in the Provisional Application No. 60/067,308, upon which this application relies for priority, and which is incorporated by reference.

It was also noticed that a "24-bp double-stranded oligonucleotide" is referred to, which includes SEQ ID No. 1 and its complementary sequence. However, SEQ ID No. 1 is actually only 23 bases in length (counting the omitted "A"). Thus, the "24" has been changed to --23-- in the specification.

Thus, this Preliminary Amendment corrects those minor typographical errors.

If there is any fee due in connection with the filing of this Preliminary Amendment, please charge the fee to our Deposit Account No. 06-0916.

Docket # 3495-0178-01

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Respectfully submitted,

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Dated: October 10, 2001

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Application Number: Not yet assigned
Filing Date: Concurrently herewith
Attorney Docket Number: 3495-0178-01

APPENDIX TO PRELIMINARY AMENDMENT

Version with Markings to Show Changes Made

Amendments to the Specification

Page 1, the first paragraph is amended as follows:

This regular U.S. application [is based on and] is a division of U.S. Application Serial No. 09/203,681 filed December 1, 1998, and claims the benefit of U.S. Provisional patent application serial No. 60/067,308, filed December 4, 1997, the entire disclosure of each of which is relied upon and incorporated by reference herein.

Page 43, the paragraph beginning at line 11 is amended as follows:

Plasmid pUT18 (3023-bp) is a derivative of the high copy number vector pUC19 (expressing an ampicillin resistance selectable marker and compatible with pT25 or pKT25) that encodes the T18 fragment (amino acids 225 to 399 of CyaA). In a first step, we constructed plasmid pUC19L by inserting a [24-bp] 23-bp double-stranded oligonucleotide [(5'-ATTCATCGATATAACTAAGTAA-3' [SEQ ID No.: 1])] (5'-AATTCATCGATATAACTAAGTAA-3' (SEQ ID No.: 1)) and its complementary sequence) between the *Eco*RI and *Nde*I sites of pUC19. Then, a 534-bp fragment harboring the T18 open reading frame was amplified by PCR (using appropriate primers and pT18 as target DNA) and cloned into pUC19L digested by *Eco*RI and *Cla*I (the appropriate restriction sites were included into the PCR primers). In the resulting plasmid, pUT18, the T18 open reading frame is fused in frame downstream of the

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multicloning site of pUC19. This plasmid is designed to create chimeric proteins in which a heterologous polypeptide is fused to the N-terminal end of T18 (see map).

Amendments to the Claims

Claims 10-22 and 25-38 are amended as follows:

10. (AMENDED) A method of selecting a molecule of interest, which is capable of binding to a target ligand, wherein the interaction between the [said] molecule of interest and the [said] target ligand is detected with a signal amplification system [according to any one of the claims 1 to 9] comprising:

(a) a first chimeric polypeptide comprising a first fragment of an enzyme chosen from adenylate cyclase and guanylate cyclase;

(b) a second chimeric polypeptide comprising a second fragment of an enzyme chosen from adenylate cyclase and guanylate cyclase, or a modulating substance capable of activating adenylate cyclase or guanylate cyclase, and

(c) a signal molecule precursor,

wherein the first fragment is fused to a molecule of interest, and the second fragment or the modulating substance is fused to a target ligand, and wherein the activity of the enzyme is restored by the *in vivo* interaction between the molecule of interest and the target ligand and wherein a signal amplification is generated by the restored enzyme activity; and

wherein the *in vivo* interaction occurs in a bacterial cell

[, by means of generating a signal amplification and triggering transcriptional activation].

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11. (AMENDED) The method of selecting a molecule of interest according to claim 10, wherein the signal amplification corresponds to [the] production of a [signaling] signal molecule.

12. (AMENDED) The method of selecting a molecule of interest according to claim 10, wherein the signal amplification triggers transcriptional activation [leads to a reporter gene expression].

13. (AMENDED) The method of selecting a molecule of interest according to [any one of claims] claim 10 [to 12], wherein the signal amplification system comprises a bacterial multi-hybrid system of at least two distinct fragments of an enzyme[, whose enzymatic activity is restored by the interaction between the said molecule of interest and the said target ligand].

14. (AMENDED) The method of selecting a molecule of interest according to [any one of claims] claim 10 [to 12], wherein the signal amplification system comprises a bacterial multi-hybrid system of at least a first fragment of an enzyme and a modulating substance[, whose activity is restored by the interaction between the said molecule of interest and the said target ligand].

15. (AMENDED) The method of selecting a molecule of interest according to [any one of claims] claim 10 [to 14], wherein the target ligand is selected from [the group consisting of protein] proteins, [peptide] peptides, [polypeptide] polypeptides, [receptor] receptors, [ligand] ligands, [antigen] antigens, [antibody] antibodies, DNA binding [protein] proteins, [glycoprotein] glycoproteins, [lipoprotein] lipoproteins, and recombinant [protein] proteins.

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16. (AMENDED) The method of selecting a molecule of interest according to [any one of claims 10 to] claim 15, wherein the molecule of interest is capable of interacting with the target ligand [and possible of binding to said target ligand].

17. (AMENDED) The method of selecting a molecule of interest according to [any one of claims 10 to 16] claim 11, wherein the interaction between the molecule of interest and the target ligand is [detected, by means of signal amplification which triggers transcriptional activation, and is] quantified by measuring [the] synthesis of the [signaling] signal molecule [or the expression of the reporter gene].

18. (AMENDED) The method of selecting a molecule of interest according to [any one of claims] claim 11, wherein the [signaling] signal molecule [corresponds to the synthesis of] comprises cAMP.

19. (AMENDED) The method of selecting a molecule of interest according to claim 11, wherein the [signaling] signal molecule [corresponds to the synthesis of] comprises cGMP.

20. (AMENDED) The method of selecting a molecule of interest according to claim [12] 46, wherein the reporter gene [expression] is selected from [the group consisting of gene] genes coding for a nutritional marker [such as lactose, maltose; gene], genes conferring resistance to antibiotics [such as ampicillin, kanamycin or tetracycline; gene], genes encoding [for] a toxin[;], genes encoding a color marker [such as fluorescent marker of the type of the Green Fluorescent Protein (GFP); gene], genes encoding [for] a phase receptor [proteins] protein or fragment thereof [such as phage receptor proteins or fragment thereof such as phage λ receptor, *lamB*], and any other gene giving a selectable phenotype.

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21. (AMENDED) The method of selecting a molecule of interest according to [any one of claims] claim 10 [to 20], wherein the molecule of interest is a mutant molecule compared to [the] a known wild type molecule and said molecule of interest is tested for its capacity [of interacting] to interact with the target ligand.

22. (AMENDED) The method of selecting a molecule of interest according to [any one of claims] claim 10 [to 21], wherein the selection is performed in [and] an *E. coli* strain[,] or in any bacterial strain deficient in endogenous adenylate cyclase or guanylate cyclase [or any other eukaryotic cell].

25. (AMENDED) A method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest, wherein [respectively] the stimulating or the inhibiting [activity] is detected with a signal amplification system comprising:

(a) a first chimeric polypeptide comprising a first fragment of an enzyme chosen from adenylate cyclase and guanylate cyclase;

(b) a second chimeric polypeptide comprising a second fragment of an enzyme chosen from adenylate cyclase and guanylate cyclase, or a modulating substance capable of activating adenylate cyclase or guanylate cyclase, and

(c) a signal molecule precursor,

wherein the first fragment is fused to a molecule of interest, and the second fragment or the modulating substance is fused to a target ligand, and wherein the activity of the enzyme is restored by the *in vivo* interaction between the molecule of interest and the target ligand and wherein a signal amplification is generated by the restored enzyme activity; and

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wherein the *in vivo* interaction occurs in a bacterial cell,

[according to any one of the claims 1 to 9, by means of generating an amplification and respectively of triggering or of abolishing transcriptional activation, and wherein said signal amplification and said triggered or abolished transcriptional activation are compared with those obtained from an identical signal amplification system without any substance] and wherein the activity of the enzyme is tested in the presence and absence of the test substance.

26. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 25, wherein the signal amplification system comprises a bacterial multi-hybrid system of at least two distinct fragments of an enzyme[, whose enzymatic activity is restored by the interaction between the said molecule of interest and the said target ligand].

27. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to [claims] claim 25, wherein the signal amplification system comprises a bacterial multi-hybrid system of at least a first fragment of an enzyme and a modulating substance[, whose activity is restored by the interaction between the said molecule of interest and the said target ligand].

28. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to [any one of claims] claim 25 [to 27], wherein the signal amplification corresponds to [the] production of a [signaling] signal molecule.

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29. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to [any one of claims 25 to 27] claim 28, wherein the signal amplification corresponding to the production of a signaling molecule is blocked or partially abolished.

30. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to [any one of claims] claim 25 [to 28], wherein the signal amplification generated by the restored enzyme activity leads to transcriptional activation, which leads to [a] reporter gene expression.

31. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to [any one of claims 25 to 27 and to claim 29] claim 30, wherein the transcriptional activation leading to [a] reporter gene expression is blocked or partially abolished.

32. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to [any one of claims] claim 25 [to 31], wherein the target ligand is selected from [the group consisting of receptor] receptors, [ligand] ligands, [antigen] antigens, [antibody] antibodies, DNA binding [protein] proteins, [glycoprotein] glycoproteins and [lipoprotein] lipoproteins.

33. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of

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interest according to [any one of claims] claim 25 [to 32], wherein the test substance is selected from [the group consisting of protein] proteins, [glycoprotein] glycoproteins, [lipoprotein] lipoproteins, [ligand] ligands, and any other [drug] compound having stimulating or inhibitory affinity.

34. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 28 [or 29], wherein the [signaling] signal molecule corresponds to [the synthesis of] cAMP.

35. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 28 [or 29], wherein the [signaling] signal molecule corresponds to [the synthesis of] cGMP.

36. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 30 [or 31], wherein the reporter gene [expression] is selected from [the group consisting of gene] genes coding for a nutritional marker [such as lactose, maltose; gene], genes conferring resistance to antibiotics [such as ampicillin, kanamycin or tetracycline; gene], genes encoding [for] toxin[;], genes encoding a color marker [such as fluorescent marker of the type of the Green Fluorescent Protein (GFP); gene], genes encoding [for] phage receptor proteins or a fragment thereof [such as phage λ receptor, *lamB*], and any other gene giving a selectable phenotype.

37. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of

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interest according to [any one of claims] claim 25 [to 36], wherein the molecule of interest is a mutant molecule compared to [the] a known wild type molecule and said molecule of interest is tested for its capacity of interacting with the target ligand.

38. (AMENDED) The method of screening for a test substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to [any one of claims] claim 25 [to 37], wherein the screening is performed in an *E. coli* strain[,] or in any bacterial strain deficient in endogenous adenylate cyclase or guanylate cyclase [or any other eukaryotic cell].

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Attorney Docket No. 3495-0178-01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Ladant et al.	)	Group Art Unit for parent application:
	)	1645
Serial No.: Not yet assigned	)	
	)	Examiner in parent application: Zeman
Filed: Concurrently herewith	)	
	)	
For: BACTERIAL MULTI-HYBRID SYSTEM AND APPLICATIONS THEREOF	)	

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

REQUEST FOR APPROVAL OF DRAWING CHANGE

Subject to the approval of the Examiner, it is respectfully requested that Figures 2 and 3.2 in the above-captioned application be amended by insertion of sequence identifiers. The changes are indicated in red on the attached copy of the originally filed drawing.

Applicants also respectfully request, subject to the Examiner's approval, substitution of Substitute Figure 3.1 for Figure 3.1 of the application as filed, with insertion of sequence identifiers as indicated in red in the drawing. The Substitute Figure 3.1 corrects a typographical error in the sequence, as described in the attached Preliminary Amendment.

Also submitted herewith is a Submission of Formal Drawings, which incorporate all of the aforementioned changes.

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Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
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Dated: October 10, 2001

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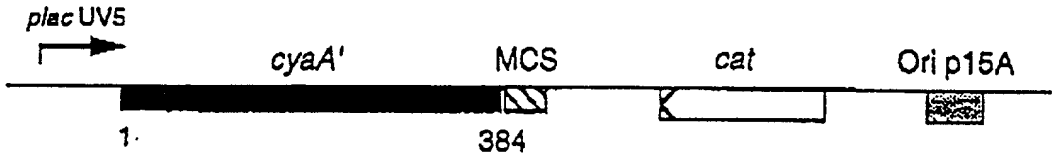
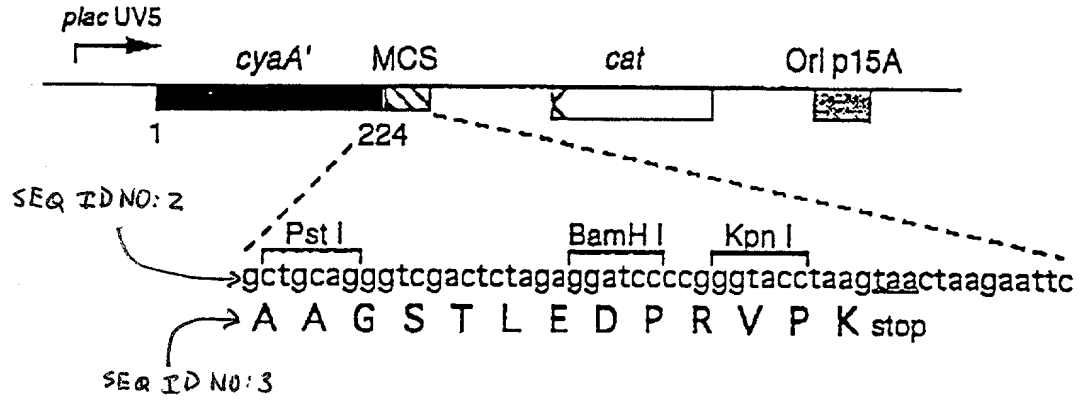
pCmAHL1



pT25



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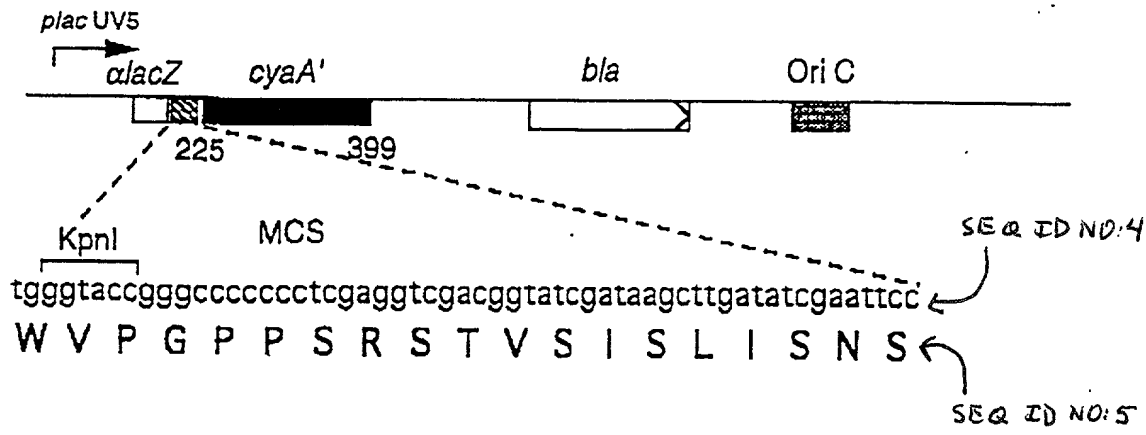
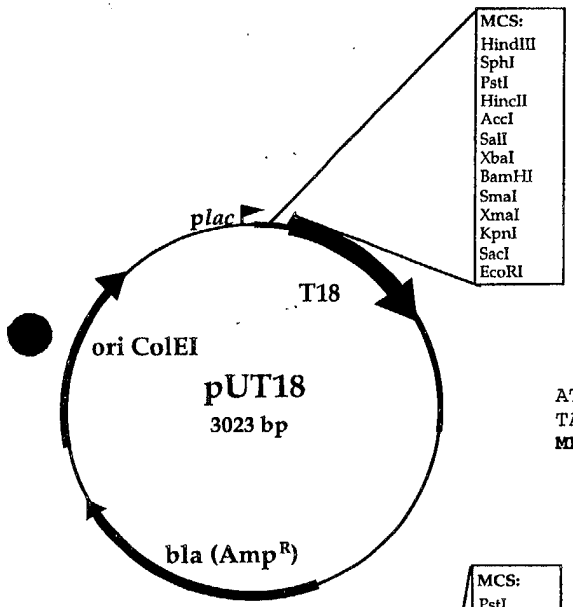
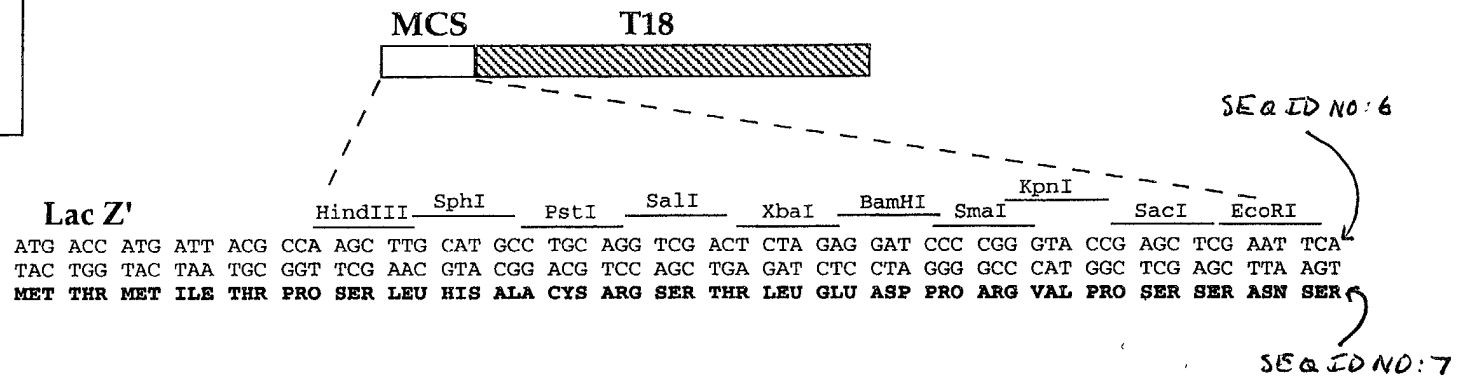
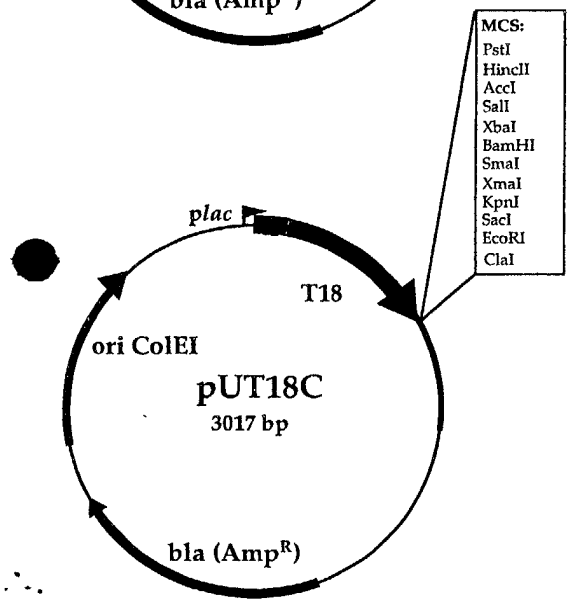


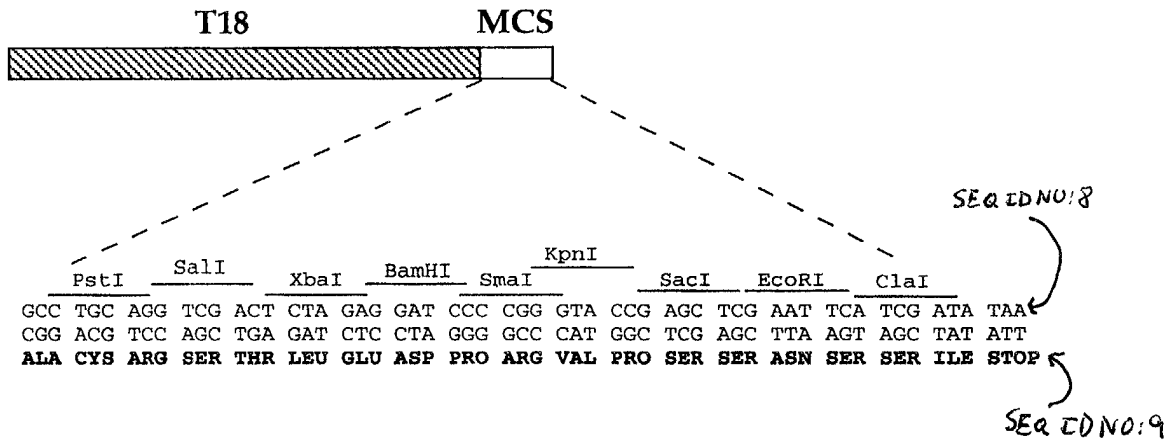
FIG. 2

VECTORS EXPRESSING THE T18 FRAGMENT



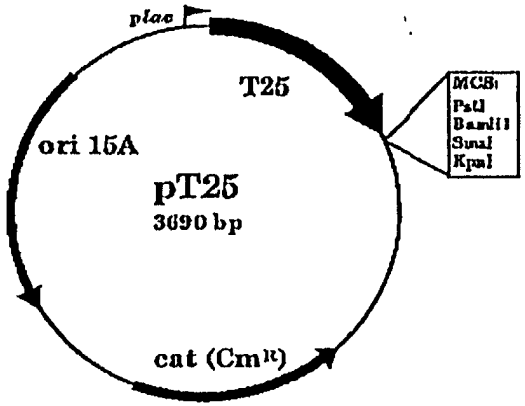




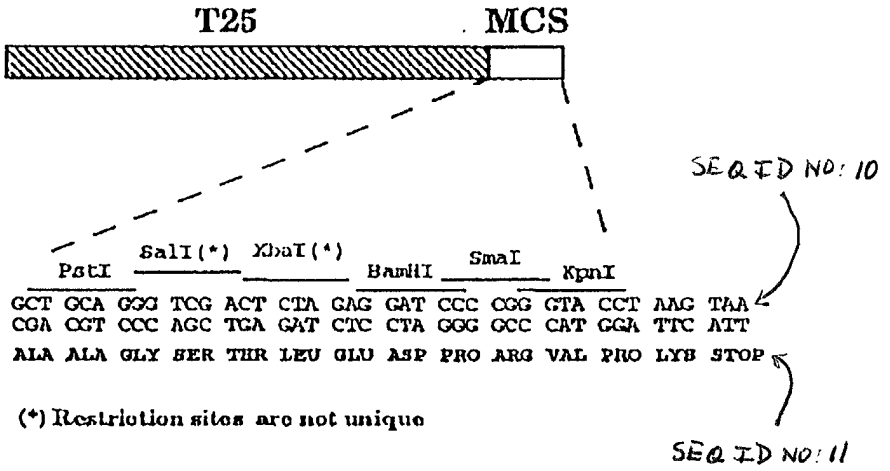


Substitute Fig. 3.1

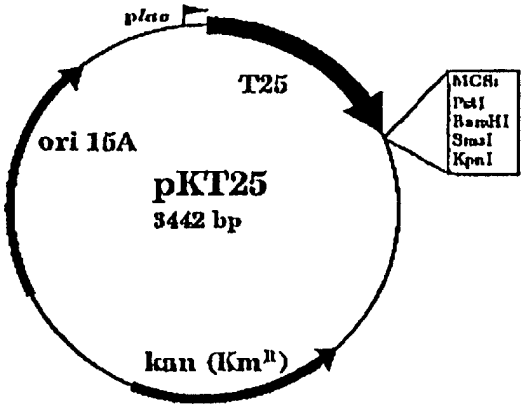
VECTORS EXPRESSING THE T25 FRAGMENT



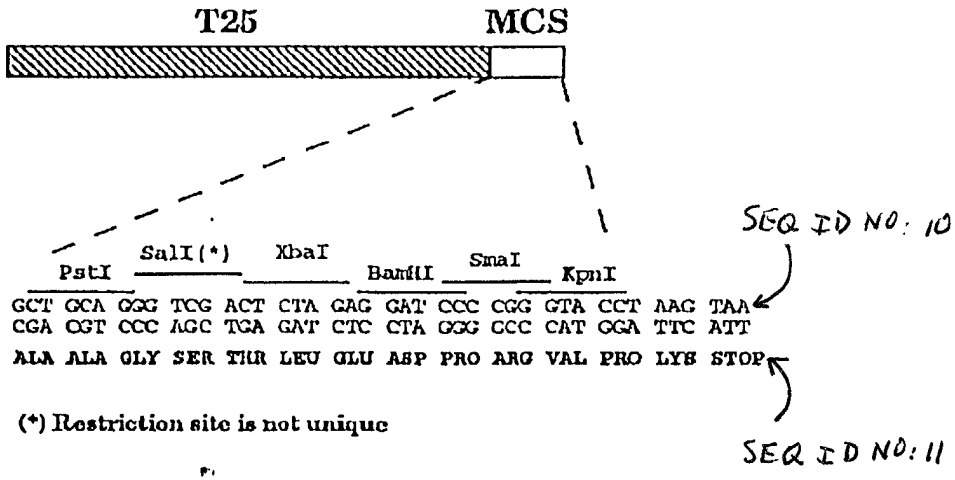
Derivative of pACYC184



(*) Restriction sites are not unique



Derivative of p8U40



(*) Restriction site is not unique

FIG. 3.2